



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/FI95/00198 <b>(22) International Filing Date:</b> 10 April 1995 (10.04.95)  <b>(30) Priority Data:</b> 941631 8 April 1994 (08.04.94) FI 945718 5 December 1994 (05.12.94) FI  <b>(71) Applicant (for all designated States except US):</b> VALTION TEKNILLINEN TUTKIMUSKESKUS [FI/FI]; Vuorim- iehentie 5, FIN-02150 Espoo (FI).  <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> ITÄVAARA, Merja [FI/FI]; Valtion Teknillinen Tutkimuskeskus, Vuorimiehentie 5, FIN-02150 Espoo (FI).  <b>(74) Agent:</b> PAPULA REIN LAHTELA OY; P.O. Box 981, (Fredrikinkatu 61A), FIN-00101 Helsinki (FI).		<b>(81) Designated States:</b> CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PROCEDURE FOR MEASURING BIODEGRADABILITY OF A SAMPLE  <b>(57) Abstract</b>  The invention concerns a procedure for measuring the biodegradability of a sample, the sample being placed in a culture solution, the solution being aerated, the quantity of carbon dioxide liberated from the solution being determined, and the biodegradability being determined on the basis of the liberated carbon dioxide quantity. As taught by the invention, the carbon dioxide which has been formed is conducted into a non-precipitating alkali solution, the electrical conductivity of this solution is measured, and the quantity of carbon dioxide absorbed in the solution is determined on the basis of electrical conductivity.		

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## PROCEDURE FOR MEASURING BIODEGRADABILITY OF A SAMPLE

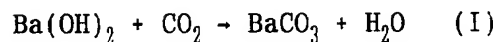
5           The present invention concerns a procedure for measuring biodegradability of a sample, the sample being placed in a culture solution, the solution being aerated, the quantity of carbon dioxide liberated from the solution being measured, and biodegradability being determined on the basis of the carbon di-  
10           oxide quantity that was liberated.

          With continuously increasing use of plastics, their disposal has become a serious problem. The rate and degree of biodegradation determine how fast, and to what degree, the materials are degraded in a microbiological environment consistent  
15           with the testing method. The results obtained by the testing method which is used help in assessing the grade of biodegradation and the time in which the plastics are potentially degraded under aerobic conditions, e.g. in effluent purifying plants.

          As a rule, in biodegradability measuring methods the  
20           quantity of carbon dioxide generated from the sample in biological degradation is determined, as a function of time, and finally the quantity of organic carbon dissolved in the nutrient solution, and the grade and rate of biodegradation of the polymer can be determined on their basis.

25           In the Sturm test, known as a standard procedure, the sample is placed in a culture solution, e.g. in a test jar, and air is conducted into the jar through a pipeline system. Prior to conducting the air into the test jar containing culture solution, the carbon dioxide present in the air is removed, e.g. by absorb-  
30           ing it in sodium hydroxide solution. Thereby the carbon dioxide which is formed in the culture solution and which can be measured by the procedure is mainly derived from biological degradation of the sample. In the system, a plurality of culture solution jars can be used in parallel. From each culture solution jar, the CO<sub>2</sub>  
35           that has formed is conducted into alkali solutions. There are three consecutive alkali solutions in the standard. When the carbon dioxide comes into contact with barium hydroxide, barium car-

bonate is formed in accordance with formula (I):



5       The quantity of carbonate produced, and thus the quantity of carbon dioxide produced in the bioreactor, is determined from the barium hydroxide solution by titration, as a function of time.

          The Sturm test is encumbered by several problems. The continuous aeration through several solutions and employing long  
10       pipelines may give rise to leakage flows; this will in turn cause inaccuracy of the results of measurement. Furthermore, a consequence of continuous aeration is generation of over-pressure within the system, and on termination of aeration reflux tends to set in, and the culture solution may enter the pipeline systems  
15       as well as other flasks. This may totally inhibit the test. The titrations comprised in the Sturm test take a lot of time, and the test is time-consuming.

          The object of the invention is to eliminate the drawbacks mentioned.

20       The specific object of the invention is to disclose a novel procedure for measuring biodegradability of samples which can be implemented more rapidly, with greater positivity and with less effort than before.

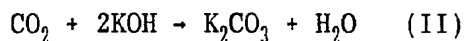
          It is a specific object to develop the procedure so  
25       that the quantity of carbon dioxide formed in the test can be measured more rapidly, preferably without employing time-consuming titration methods.

          It is a further object to develop the procedure so  
30       that the result of measurement, i.e., the biodegradability of the sample, can be determined automatically, as rapidly as possible and reproducibly.

          The invention is characterized by that which is stated in the claims.

35       The procedure of the invention is based on continuous aeration of the culture solution in the bioreactor. The carbon dioxide present in the input air is removed. The carbon dioxide produced in microbial activity is recovered by conducting the gas

coming from the bioreactor into a jar containing non-precipitating alkali solution. For alkali solution, any non-precipitating alkali solution can be used at suitable concentrations, e.g. NaOH, KOH, etc. The carbon dioxide that was produced will react with the alkali solution, producing carbonate and water in accordance with reaction (II), where KOH is stipulated as alkali solution:



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As a result of the reaction the electrical conductivity of the solution decreases. The decrease of electrical conductivity is observed with the aid of a sensor placed in the KOH jar, for instance, and the quantity of carbon dioxide contained in the gas conducted into the KOH solution is determined from the measurement readings, as a function of time. - If desired, the quantity of carbon dioxide conducted into the KOH solution may also be determined chemically, e.g. by titration, e.g. using HCl solution, with phenolphthalein for indicator, in accordance with the reaction (III):

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The quantity of carbon dioxide which has come into the KOH solution can be calculated by formula (IV):

25

$$m_{\text{CO}_2} = \Delta V_{\text{HCl}} * C_{\text{HCl}} + (V_{\text{KOH}}/V_{\text{sample}}) * M_{\text{CO}_2}, \quad (\text{IV})$$

where

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$\Delta V_{\text{HCl}}$  = Volume of HCl consumed compared with the initial situation (in ml),

$C_{\text{HCl}}$  = Molality of HCl used in titration (in mmol/ml),

$V_{\text{KOH}}$  = Volume of potassium hydroxide solution (in ml),

$V_{\text{sample}}$  = Volume of KOH sample used in titration (in ml),

35

$M_{\text{CO}_2}$  = Molar weight of carbon dioxide, 44 g/mol.

As taught by the invention, the quantity of carbon

dioxide bound in the alkali solution is determined from the electrical conductivity of the solution. For alkali solution, any non-precipitating alkali solution can be used, e.g. alkali hydroxides (NaOH, KOH, LiOH) or ammonium hydroxide. Measurement of electrical conductivity can be implemented in a way known in itself in the art, using for sensors any appropriate sensors, advantageously platinum electrodes. The use of electrodes is particularly advantageous in that measurement is rapid and accurate, and the results of measurement are obtained in the form of electrical signals. Further processing of the signals can be implemented in the way known in itself from electrotechnics, and the signals can after conversion to desired form be transferred directly to a calculating device, e.g. a micro-computer, by means of which the total quantity or the rate of production of the formed carbon dioxide can automatically be determined as a function of time.

The procedure of the invention facilitates determinations of biodegradability, because no titration is required in the new procedure.

Thanks to the invention, large numbers of samples can be simultaneously examined; the procedure reduces the determination costs.

The invention is in the following described in detail, referring to the attached drawings, wherein

Fig. 1 presents an apparatus for implementing an embodiment of the procedure of the invention;

Fig. 2 illustrates the calibration curve of the electrical conductivity sensors, created with the apparatus of Fig. 1;

Fig. 3 displays carbon dioxide contents of samples subjected to determination by the procedure of the invention, determined by different methods;

Fig. 4 displays the effect of active carbon filtration of the formed CO<sub>2</sub> on biodegradability results;

Fig. 5 presents, as a function of time, the quantity (in mg) of carbon dioxide formed in the procedure of the invention in a biodegradability test of  $\beta$ -hydroxybutyrate/valerate;

Fig. 6 presents the means obtained in parallel tests for the car-

bon dioxide quantity formed in the procedure of the invention in a biodegradability test of  $\beta$ -hydroxybutyrate/valerate;

Fig. 7 presents, as a function of time, the quantity of carbon dioxide formed in the procedure of the invention in a biodegradability test of  $\beta$ -hydroxybutyrate/valerate (the so-called null sample subtracted); and

Fig. 8 presents the biodegradability determined from the samples by the procedure of the invention, as a function of time.

Fig. 1 shows, schematically, an apparatus for implementing the procedure of the invention. A pump 1 has been disposed to pump air, for use in aeration, through containers 2 containing silicagel and into absorption tubes 3, where the carbon dioxide present in the air used for aeration is removed by the aid of absorption. The absorption tubes 3 may contain e.g. any carbon dioxide-eliminating substance whatsoever, such as a hydroxide, e.g. sodium hydroxide granules. After the four absorption tubes placed (two and two) in series and in parallel, the aerating gas is conducted through consecutive jars 4 containing e.g. 0.1 M barium hydroxide and to the measuring units proper, 5. This makes sure of the  $\text{CO}_2$  removal and indicates it, and it humidifies the aeration air. In Fig. 1 only two measuring units 5, of altogether eight, have been depicted.

The measuring unit 5 comprises a bioreactor 6, in which the sample to be examined is placed. In the bioreactor culture solution has been placed, capacity of the bioreactor e.g. 2000 ml, quantity of culture solution e.g. 1000 ml. The gas forming in the bioreactor is conducted into a measuring jar 6 containing 0.1 M KOH solution, and in which has been placed a measuring sensor, that is an electrode 7 for determining the carbon dioxide bound in the KOH solution, on the basis of the KOH solution's electrical conductivity. The gas is conducted from the measuring unit 6 to another jar 9 containing KOH solution, to make sure of the  $\text{CO}_2$  recovery. Each one of the jars 6 and 9 contains 300 ml of 0.1 M KOH solution. This solution quantity, and concentration, is sufficient to bind 660 mg of carbon dioxide.

The electrode placed in the measuring jar 6 is connected to a switch array 10 and, further, to a data gathering

device 11 for processing and measuring the voltage signals. Furthermore, the signals obtained from the switch array 10 and from the data gathering device 11 are carried to a dedicated computer 12, for processing the results and calculating the final results.

5           The bioreactor 6 is advantageously fitted with a check valve 13, which prevents the culture solution from entering the pipeline system 20 by effect e.g. of pressure fluctuations or other causes.

#### 10       Example 1: Calibrating the sensors

          Calibration of the sensors is performed e.g. by the method described by Chapman (1971), according to which calibration solutions were prepared by mixing KOH and  $K_2CO_3$  solutions in various proportions. 0.1 M KOH and 0.05 M  $K_2CO_3$  solutions are  
15       mixed so that the degree of saturation of the completed solutions with regard to potassium carbonate varies in the range from 0 to 100%; the volume of the solutions was 300 ml.

          The sensors were placed in the calibration solutions and the voltage number reflecting the electrical conductivity of  
20       the solutions was measured. Measurement was automatically accomplished at 10-minute intervals during 24 hours, in which time the sensors settled at a constant value. Upon stabilization, the mean was calculated of the last ten results of measurement, which was taken to be the calibration value of the sensor at the respective  
25       degree of saturation of the calibration solution. The calibration solutions were swapped from sensor to sensor, the sensors were allowed to stabilize, and voltage readings were recorded. This was repeated until calibration values had been obtained with all calibration solutions for all sensors. Fig. 2 shows the results  
30       of measurement of the calibration graphs found in this way.

          The electrical conductivity of a solution is known to depend on the temperature of the solution. For finding out the influence of temperature, the voltage reading reflecting the electrical conductivity of two different calibration solutions  
35       (degree of saturation 0% and 50%, respectively) was measured as a function of temperature. According to the results, the voltage readings were found to decrease only 0.00487 V (0% sat.) and



0.00714 V (50% sat.) per 1° increment of temperature. In the experimental conditions the temperature of the KOH solutions has been found to fluctuate less than 0.1°C, and the effect of temperature on the voltage readings could therefore be neglected.

5

#### Example 2: Carbon dioxide measurements

Recovery of carbon dioxide and operation of the electrical conductivity sensors were tested using a set-up as shown in Fig. 1. Carbon dioxide was produced from sodium carbonate. A known quantity of  $\text{Na}_2\text{CO}_3$  was added into bioreactor flasks containing 1 litre of ultra-pure water. 2.0 M HCl was added into the flasks, to liberate the carbon dioxide from the carbonate. The released  $\text{CO}_2$  was conducted along with aeration air into flasks containing 0.1 M KOH. From the flasks the quantity of recovered  $\text{CO}_2$  was determined on the basis of change of electrical conductivity, and by titration. The results of the test are presented in Fig. 3, displaying graphically the carbon dioxide determinations based on electrical conductivity, on titration and on theoretical values.

20

The results reveal that recovery of the generated carbon dioxide is efficient. It is also noted that the results obtained by different methods are very closely equal, from which the inference can be drawn that the procedure based on change of electrical conductivity works well indeed.

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#### Example 3: Washing the $\text{CO}_2$ gas

In degradation tests, with glucose as carbon source and Escherichia coli as degrading organism, other metabolic products were also found to be formed, in addition to carbon dioxide, which increased the voltage reading when entrained in the alkali solution. The alkali solutions were analysed by mass spectrometer, and about twenty different organic compounds were found, part of them polar ones, which were considered to affect the electrical conductivity of the solution. With a view to eliminating these compounds, various ways of washing the gas coming from the bioreactor were tried out. Washing of the gas was tried with various washing fluids, yet removal of the volatile organic

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compounds affecting electrical conductivity which are contained in the gas from the bioreactor proved to be inadequate in efficiency. Therefore, purification of the gas by filtration through active carbon was tested. The test arrangement was consistent with Fig. 1 in principle. The measuring jars 4, 6 and 8 comprised active carbon filtration.

The results of this test are shown in Fig. 4. This figure reveals that in the case of measuring jar 4 all the carbon dioxide came through in active carbon filtration, and the titration and electrical conductivity results are both equal in magnitude. The active carbon filtration is seen to have worked well in measuring jars 5 to 8, the electrical conductivity results declining to the level of the results obtained by titration.

Filtration of the gas from the bioreactors with active carbon for elimination of volatile organic compounds proved to be a well-functioning procedure.

#### Example 4: Biodegradability experiment with polymers

As an example of biodegradability tests with real polymers, there shall be presented the degradation, in the procedure of the invention, of poly- $\beta$ -hydroxybutyrate/valerate (PHB/V). Biodegradation of 100, 200 and 300 mg of PHB/V was studied in the experiment.

Figs 5 to 8 present, step by step, the test carried out with apparatus based on measurement of electrical conductivity. In Fig. 5 is seen, as a function of time, the quantity of carbon dioxide formed in eight bioreactors. Left on top is the so-called null sample (microbe inoculation only; no polymer sample). Left on the bottom, the sample consists of 100 mg, right on top of 200 mg, and right on the bottom of 300 mg PHB/V per litre.

In Fig. 6 are seen the means of the replicate results in Fig. 5. These means are further used to calculate the biodegradability.

Next, the carbon dioxide yielded by the null sample jars is subtracted from the carbon dioxides of the measuring jars which contained sample material. The results are displayed in Fig. 7. Left on top refers to a sample of 100 mg, left on the

bottom to 200 mg, and right on top to 300 mg PHB/V per litre.

When the carbon quantities present in the samples have been input in the program software of the computer, the program will compute the biodegradability of the sample as a function of time. Biodegradability is the proportional fraction of carbon dioxide formed from the sample, related to the theoretical carbon dioxide formation calculated on the basis of the quantity of organic carbon present in the sample. Fig. 8 shows, as a function of time, the biodegradability of the samples in the present test. Left on top refers to a sample of 100 mg, left on the bottom to one of 200 mg, and right on top, to 300 mg PHB/V per litre.

From the results obtained by titration one may calculate, by way of example, the results relating to the sample which contained 100 mg PHB/V.

1 ml samples drawn from the alkali measuring jar consumed in one of two parallel flasks 5.2 ml less, and in the other 5.0 ml less 0.003 M HCl than what pure KOH solution consumed at the start of the test. This yields for the carbon dioxide quantity contained in the KOH measuring jars, by the formula above presented:

$5.2 \text{ ml} * 0.003 \text{ mmol/ml} * (300 \text{ ml/1 ml}) * 44 \text{ mg/mmol} = 205.9 \text{ mg},$   
respectively, 198 mg.

The mean is 201.95 mg.

Subtracting from this mean the mean, 49.4 mg, of the carbon dioxides in the null sample measuring jars we find for the carbon dioxide quantity generated from the samples: 152.55 mg. Knowing that 100 mg of PHB/V contains 57.9 mg carbon, we find for the theoretically formed carbon dioxide quantity: 212.3 mg. The biodegradability is thus found to be;

Biodegradability =  
 $(152.55 \text{ mg}/212.3 \text{ mg}) * 100\% = 71.8\%$

The biodegradability was calculated in like manner for the other samples as well. The biodegradability of all samples, calculated

from the results of titration and as results obtained with the electrical conductivity apparatus, is presented below.

5	Sample	BD percentage	BD percentage
		Titration	El. conductivity
	100 mg PHB/V	71.8	68.6
	200 mg PHB/V	77.4	81.6
	300 mg PHB/V	66.8	70.3

10 The embodiment examples are meant to illustrate the invention, without in any way confining it.

## CLAIMS

1. A procedure for measuring the biodegradability of a sample, the sample being placed in culture solution, the solution being aerated, the quantity of carbon dioxide liberated from the solution being determined, and the biodegradability being determined on the basis of the liberated carbon dioxide quantity, characterized in that the carbon dioxide which has been formed is conducted into a non-precipitating alkali solution, the electrical conductivity of this solution is measured, and the quantity of carbon dioxide absorbed in the solution is determined on the basis of electrical conductivity.

2. Procedure according to claim 1, characterized in that in the procedure the quantity of liberated carbon dioxide is determined as a function of time.

3. Procedure according to claim 1 or 2, characterized in that the changes in electrical conductivity are measured as a function of time, the results of measurement are stored in a computer, and the biodegradability of the sample is automatically determined with the aid of an EDP programme.

4. Procedure according to any one of claims 1-3, characterized in that the carbon dioxide formed in the culture solution is filtered through a suitable filter in order to remove any interfering substances which have been formed.

5. Procedure according to any one of claims 1-4, characterized in that the carbon dioxide is filtered through active carbon.

6. A procedure for measuring the biodegradability of a sample, the sample being placed in a culture solution, the solution being aerated, the quantity of carbon dioxide liberated from the solution being determined, and the biodegradability being determined on the basis of the liberated carbon dioxide quantity, characterized in that the carbon dioxide which has been formed is conducted into a non-precipitating alkali solution, the changes in electrical conductivity of this solution are automatically measured, the results of measurement are stored in a computer, and the biodegradability of the sample is automatically determined with the computer with the aid of an EDP programme.

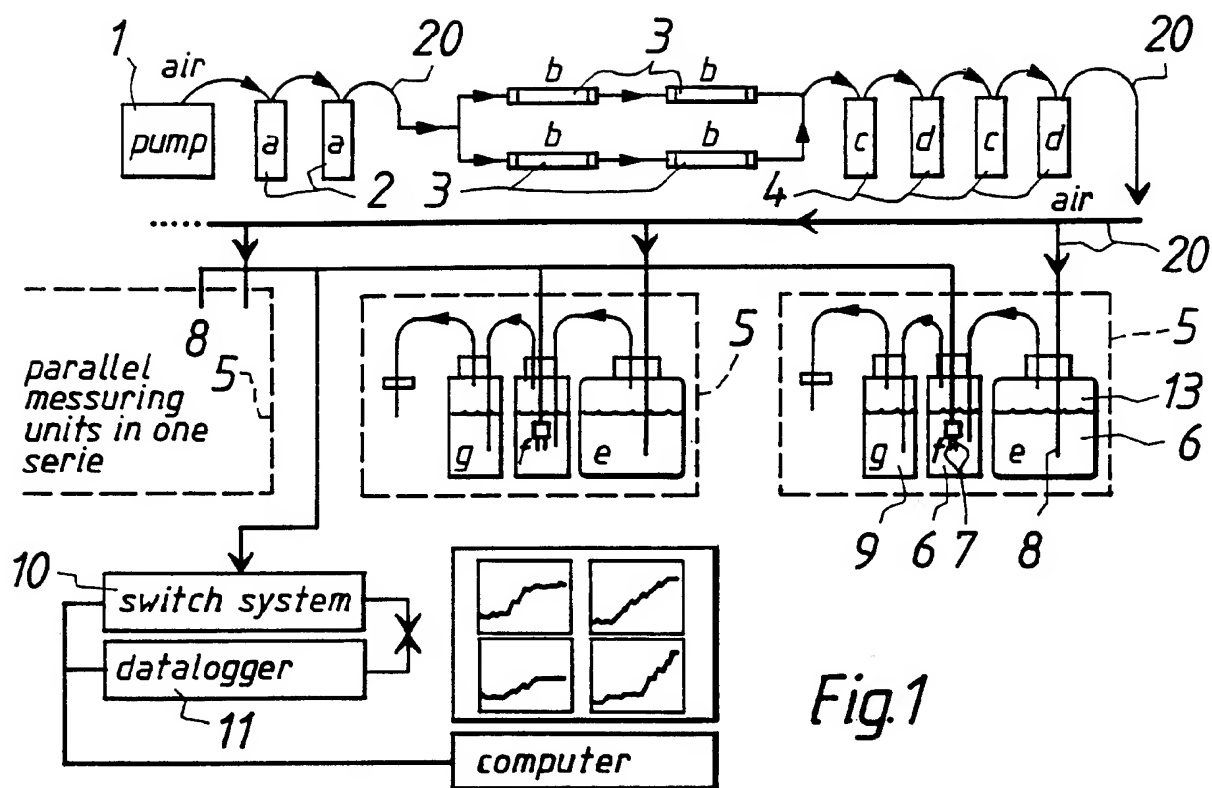


Fig. 1

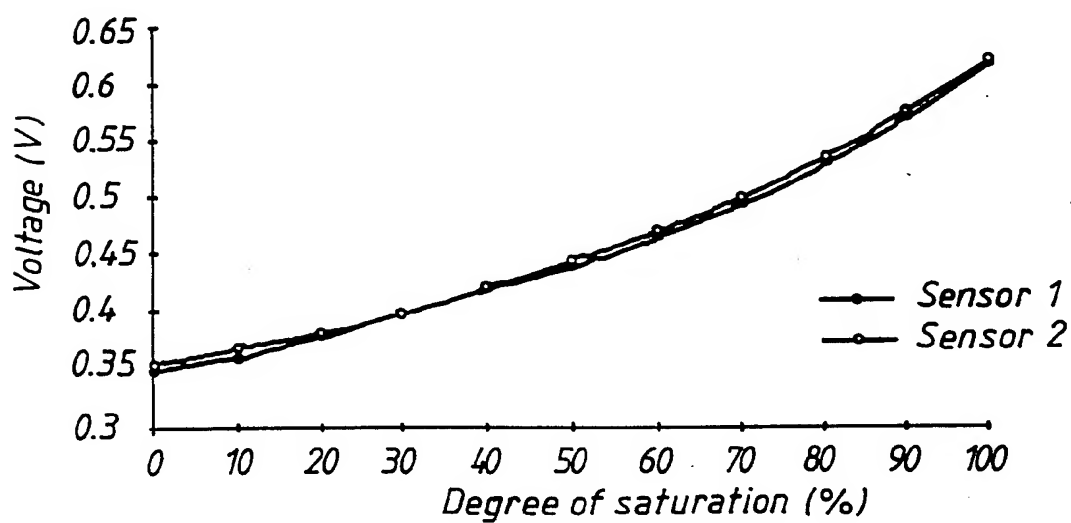


Fig. 2

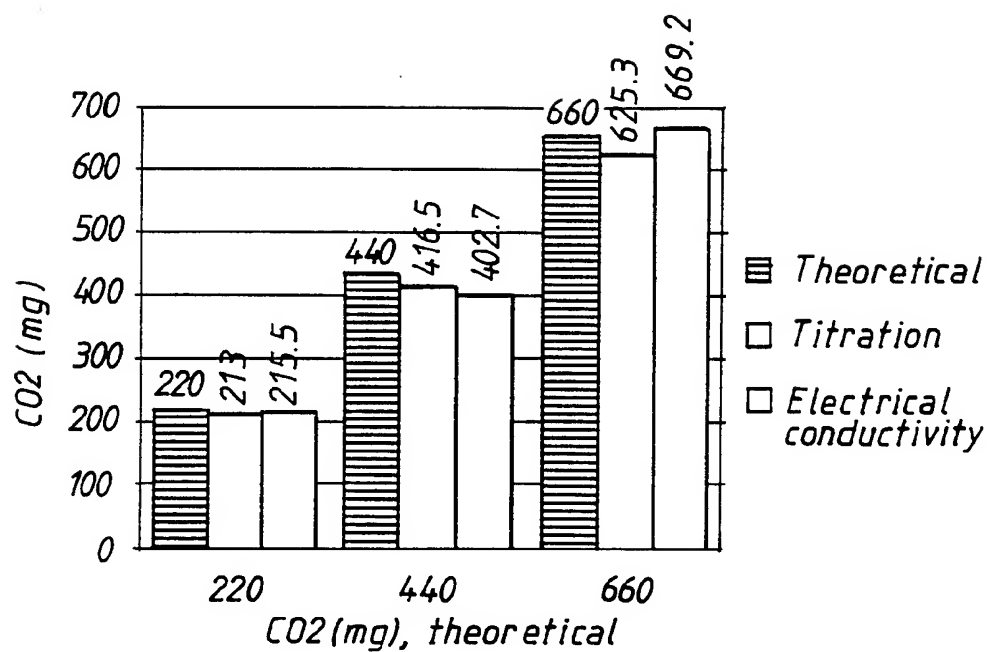


Fig. 3

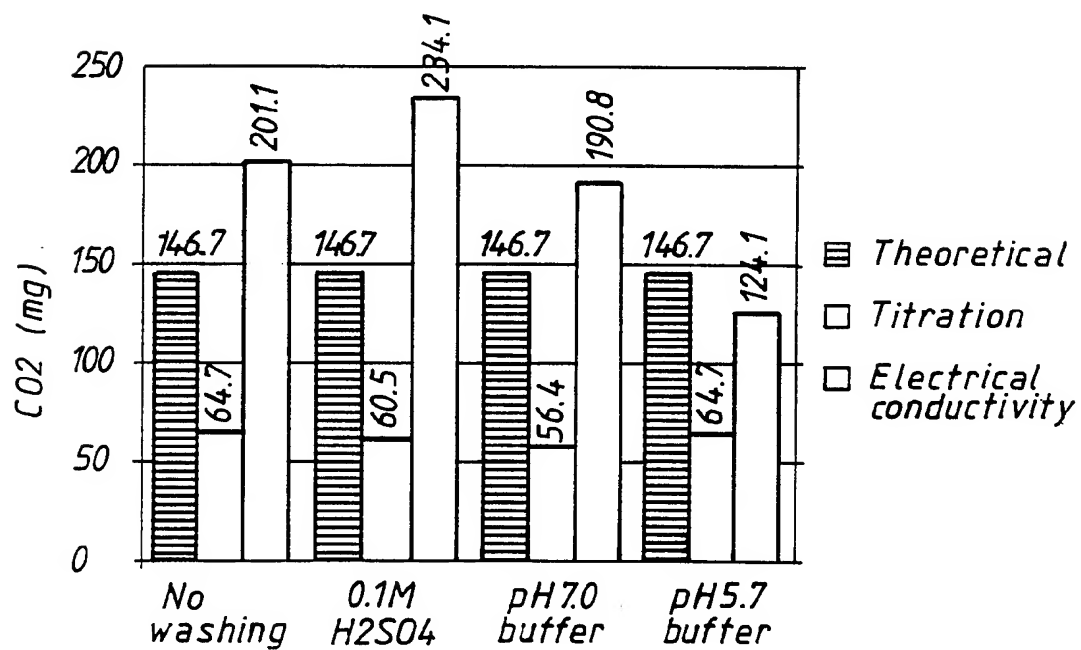
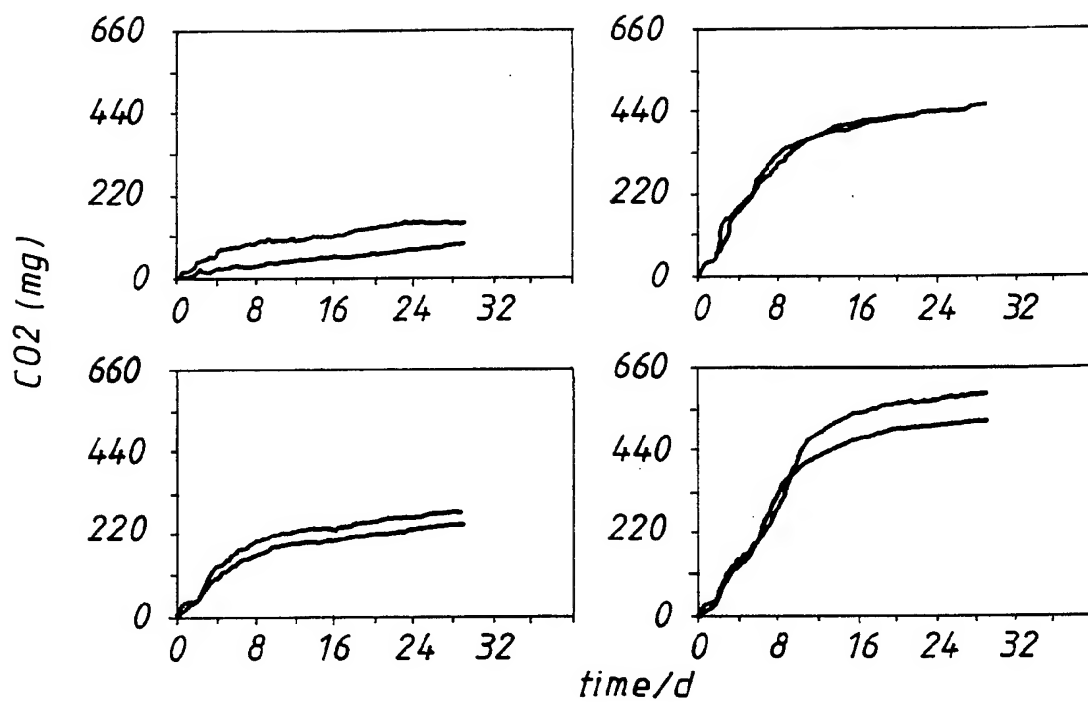
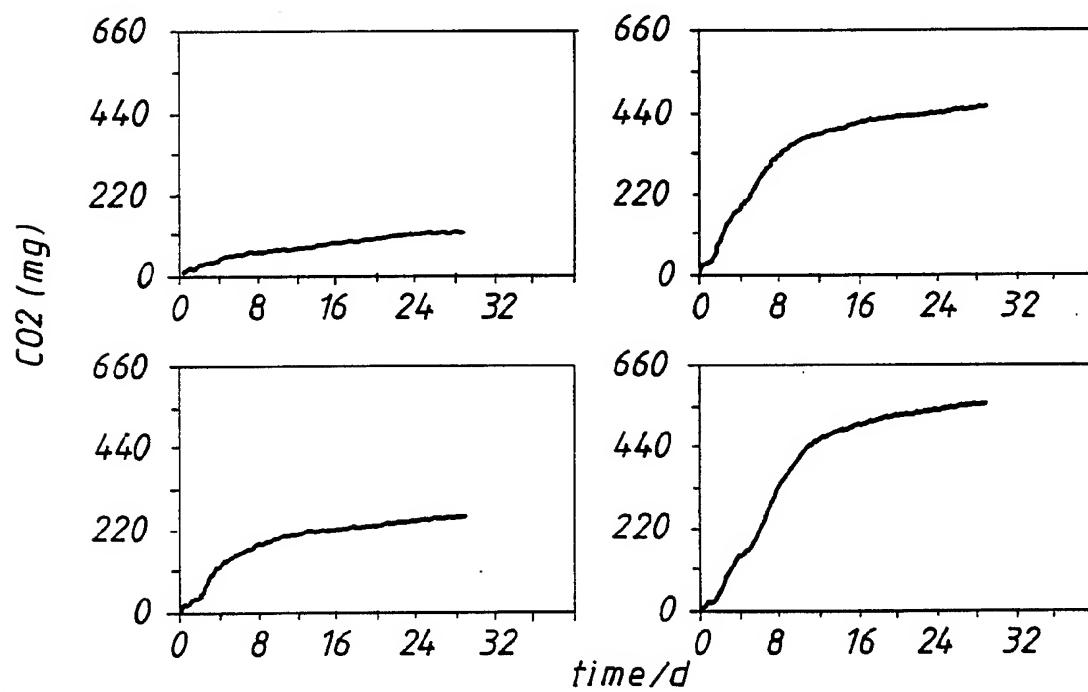


Fig. 4

*Fig.5**Fig.6*



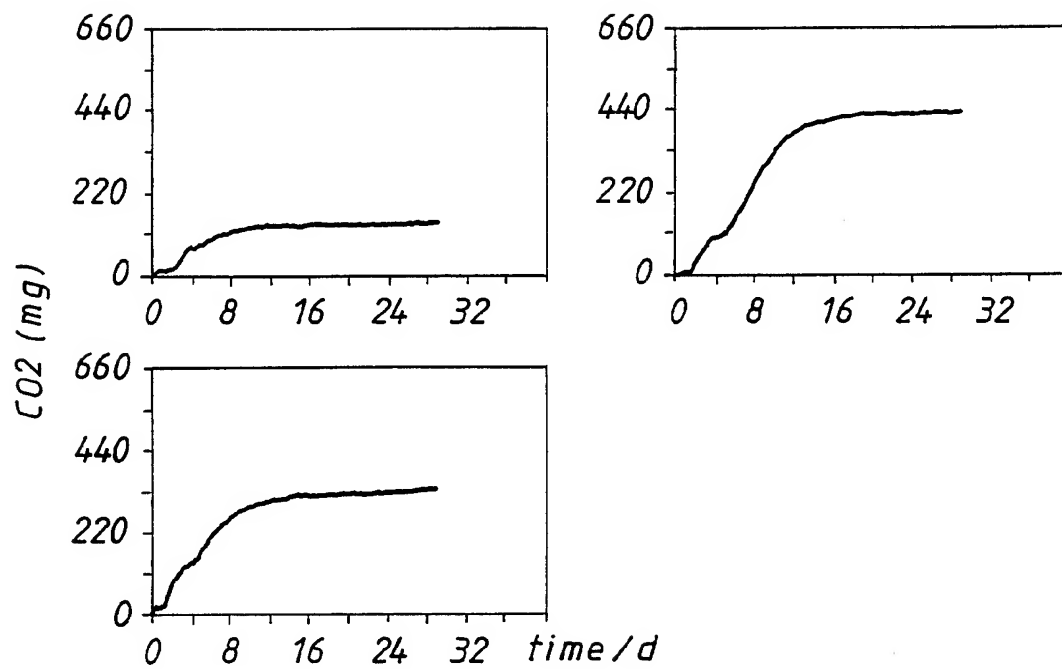


Fig.7

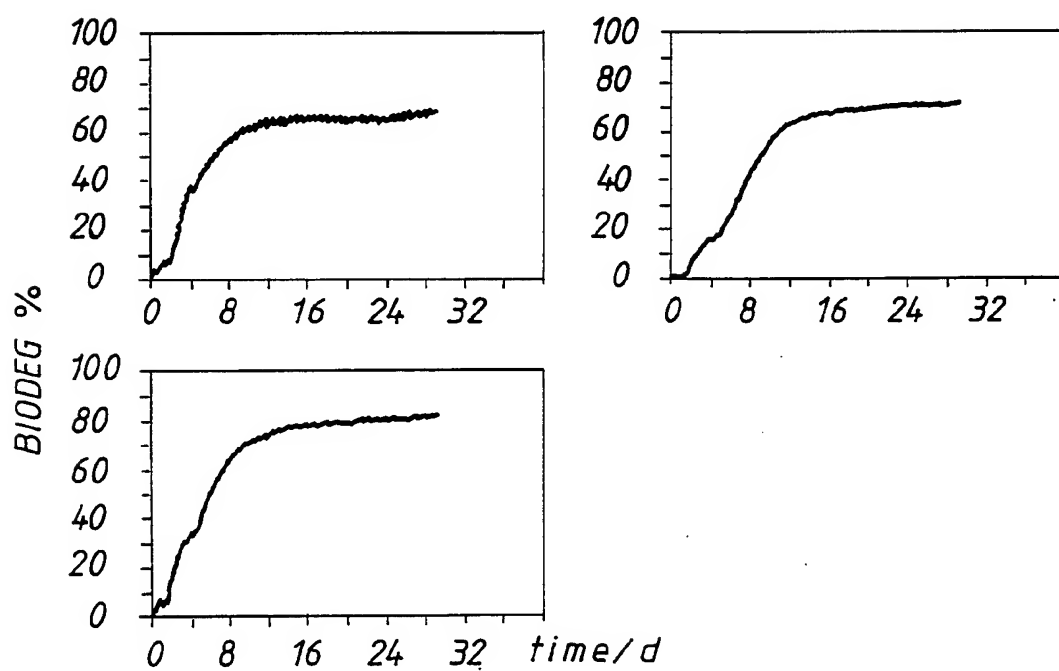


Fig.8

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 95/00198

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12Q 1/02, C12M 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, IFIPAT, CA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5278248 A (JEAN-BERNARD EGRAZ ET AL), 11 January 1994 (11.01.94)  --	1-6
A	EP 0499302 A1 (ORGANIC WASTE SYSTEMS N.V.), 19 August 1992 (19.08.92)  -- -----	1-6

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Information on patent family members

29/05/95

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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